SEROEPIDEMIOLOGIC SURVEY FOR ANTIBODY TO *BUNYAVIRUS* OF SHEEP IN TEXAS

A Thesis

Presented to

the Faculty of the Graduate School of

Angelo State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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August 1986

SEROEPIDEMIOLOGIC SURVEY FOR ANTIBODY

TO BUNYAVIRUS OF SHEEP IN TEXAS

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DEDICATION

This thesis is dedicated to the memory of my grandfather. to my parents and grandmother, I dedicate this work, with my sincerest appreciation and most gratitude for their financial and psychical supports and encouragements.

"If you decide to become a veterinary surgeon --- you will never grow rich, but you will have a life of endless interest and variety. "

--- James Herriot

ACKNOWLEDGEMENTS

I would like to acknowledge the following persons for their help in the successful completion of this thesis. Sincerest thanks to:

The Graduate Advisory Committee for carefully proofreading and providing helpful suggestions on the thesis

Dr. Crosby W. Jones Jr. for his continued supports, helps, editorial commentary efforts on my English ability and behalf, and friendship

Dr. Charles W. Livingston Jr. for his timely suggestions, encouragement, helps, and professional courtesies extended to me

The Angelo State University Biology Faculty for their suggestions and willingness to share the benifits of their experiences

Dr. Carl Menzies, Director of the Texas Agricultural Experiment Station, San Angelo, Texas (Texas A&M University System), for affording me a part-time job and the privilege of utilizing the research facilities and studying animals

Mrs. Betty Gauer for her suggestions, helps, and teaching me English as an English teacher

Mrs. Polly Crawford for her assistance in acquiring periodical literature, which would have otherwise been inaccessible; always with a smile

My uncle, K. T. Chung and aunt, Brenda Chung for their psychical support and encouraging pursuit of my interests and development of my abilities

Dr. Bernard T. Young, Graduate Dean of Angelo State University, for his encouragement and support

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SEROEPIDEMIOLOGIC SURVEY FOR ANTIBODY TO BUNYAVIRUS OF SHEEP IN TEXAS

Introduction

Two isolates of an unusual virus were isolated in 1981 from ruminants in Texas. One was from a ram showing stiffness and febrile signs and the other was from a herd of cattle with reproduction problems but with no other unusual signs of illness (19). The viral isolates were shown to be members of the Bunyamwera supergroup (family Bunyaviridae, genus Bunyavirus) by complement-fixation tests, and physical and chemical characteristics (21).

On the basis of immunological, chemical, and morphologic features, many so-called arboviruses have been grouped in a single family, Bunyaviridae. There are four genera in the family Bunyaviridae, including Bunyavirus, Phlebovirus, Nairovirus, and Uukuvirus (20). Melnick (27) included 5 genera in the family Bunyaviridae including the 4 genera described above and Hantaanvirus.

The main characteristics of the family are as follows: the virions are spherical, enveloped particles, 90-100 nm in diameter; the envelope contains two glycoproteins; the genome consists of three single-stranded segmented RNAs of a total molecular weight of 6-7 x 10^6 daltons (4, 11). Suckling and newly weaned mice are the laboratory animals of choice for isolation of Bunyaviridae viruses. The viruses produce cytopathic changes and plaques in a variety of cultured cells. Bunyaviridae viruses replicate in cell cytoplasm and are formed by budding through Golgi and endoplasmic reticulum membranes. They are accumulated within cisternae

of these organelles (22, 23). The immunologic cross-reactivity of Bunyaviridae viruses is apparent from results of hemagglutination-inhibition tests, complement fixation tests, and neutralization tests. The viruses are transmitted primarily by mosquitoes but ticks can be carriers in some instances.

Sixteen serological groups containing at least 145 viruses have been classified into genus Bunyavirus (20) making it the largest group of arboviruses. Bunyavirus was isolated first from a pool of Aedes spp. mosquitoes in Uganda in 1943 (25). Since then, bunyaviruses have been isolated from mosquitoes, human blood (25), and mammals (21). Their geographic distributions are limited to relatively narrow or specific ranges, suggesting dependence upon the distribution of their arthropod vectors and natural vertebrate hosts. Different degrees of fever, headache, encephalitis, myalgia, and hemorrhagic signs of Bunyavirus infection in humans have been reported (4). Antibodies against Bunyavirus have been detected in sera from residents of Uganda, Tongaland (17, 26), Nigeria, and Tanganyika in Africa and Malaya and Borneo in Asia (26).

The major Bunyavirus isolates in the United States are Cache Valley virus (Utah, 1956) isolated from Culiseta inornata mosquitoes (10, 25), Tensaw virus (Arkansas, 1961), strains of virus closely related to Cache Valley virus (Florida, 1962) (2, 25), and other strains of virus (Florida and Virginia, 1965) all from Anopheles crucians mosquitoes, a Cache Valley strain isolated from an apparently healthy horse in Michigan (21), viruses from mosquitoes collected in Colorado, Illinois, and New Mexico (25), and Lokern virus (12). Santa Rosa virus, Tlacotalpan virus, and Wyeomyia virus isolates originated in Mexico (12). With the

exceptions of Cache Valley virus isolated from a sick caribou in Wisconsin (8) and the Main Drain virus isolated from an encephalitic horse in California (5), there has been no outbreak of bunyavirus disease reported among domestic animals and wildlife in the United States.

Worldwide, three important Bunyaviridae virus diseases of domestic animals have been reported, as follows: Rift Valley fever [caused by genus Phlebovirus] (29), Akabane sheep disease (congenital arthrogryposis-hydranencephaly syndrome) [caused by genus Bunyavirus] (14, 30), and Nairobi sheep disease [caused by genus Nairovirus] (20). Rift Valley fever was described first in 1931 in Kenya and severe epidemics have occurred recently throughout Africa (29); it is an acute viral disease, affecting principally sheep and cattle, causing heavy mortality in young lambs and calves and abortion in pregnant ewes and cows (31). Akabane disease was isolated first in Japan and later in Australia (14). Seasonal congenital arthrogryposis-hydranencephaly syndrome in cattle, sheep, and goats has been reported in Israel (13). Spontaneous abortions and calves, lambs, and kids born dead, premature, or with deformities have been observed (13, 14). Nairobi sheep disease was reported first in 1910 between Nairobi and Mount Kenya in East Africa (1). It is characterized by an acute febrile reaction, hemorrhagic gastroenteritis, respiratory problems, abortion and usually has a mortality between 30-70% in sheep (1). Severe epizootics may occur in fully susceptible young lambs with up to 100% mortality (6).

Bunyavirus strain 7856, which was isolated in 1981 (19), is closely related to Cache Valley virus as shown by serum dilution-plaque reduction neutralization tests (21). The viral characteristics were determined and it was found that the virus was sensitive to lipid sol-

vent, that the size of the virion appeared to be between 50-100 nm by filtration and 70 nm by electron microscopy, and that the virus was heat (56C) and acid (pH=3) labile (21).

Cytopathic effects were produced in Vero, LLC-MK2, embryonic bovine testicle, and PS tissue culture cells. The inoculation of suckling or weaned mice intracranially, but not intraperitoneally produced pathogenic changes. Elevation of body temperature, depression, tremors, muscle spasms, disorientation, feeding anomalies, convulsions and other signs of central nervous system disturbance were observed in inoculated gnotobiotic and conventional sheep and goats (21). The virus is apparently pathogenic for sheep and perhaps other livestock. Since it is the first isolation in Texas of Bunyavirus from domestic ruminants, the economic losses, geographic distribution, antigenic relationships with other recognized pathogens of large mammals, and exact mode of transmission are still unclear. The purpose of this research was to survey the geographic distribution of bunyavirus neutralizing antibody among sheep in Texas in 1981.

Materials and Methods

In this study, 502 individual sheep sera were tested for bunyavirus antibody. Three hundred and ninty-eight serum samples were collected from ram lambs (6-12 months old) at the beginning of the Sonora Ram Test conducted near Sonora, Texas in 1981. This is the same year that Bunyavirus was isolated from blood samples collected from a sick ram (19) at Mertzon, Texas by research workers at the Texas A&M Agricultural Research and Extension Center at San Angelo, Texas.

During the Sonora Ram Test, 366 ram lambs representing 50 flocks from 22 counties in Texas were sampled, as were 32 ram lambs from three flocks from other states. The latter included eight ram lambs from Colorado, 21 ram lambs from Kansas, and three ram lambs from Wyoming (Figure 1 and Figure 2). The remainder of the samples (104 samples) was collected five years later (in 1986) in Texas. Of these, forty samples were from one flock in Tom Green County (including three 3-year-old rams, seven 4-month-old ram lambs, and thirty 4-month-old ewe lambs), forty samples were from another flock in Tom Green County (including three 4-month-old ewe lambs and thirty-seven 14-month-old ewes), and twenty-two samples were from a flock of 14-month old ewes in McCulloch County. Two samples brought in by a rancher were from 4-year-old ewes.

Media - The following media and reagents were used during the course of this study.

Phosphate Buffered Saline (PBS) - PBS (10x) was prepared by dissolving the following components in 1,000 ml of distilled water:

1. Sodium chloride (NaCl) ----------80.00 gm.

FIGURE 1 - Location of Counties in Texas from which Rams were Tested for Bunyavirus Neutralizing Antibody

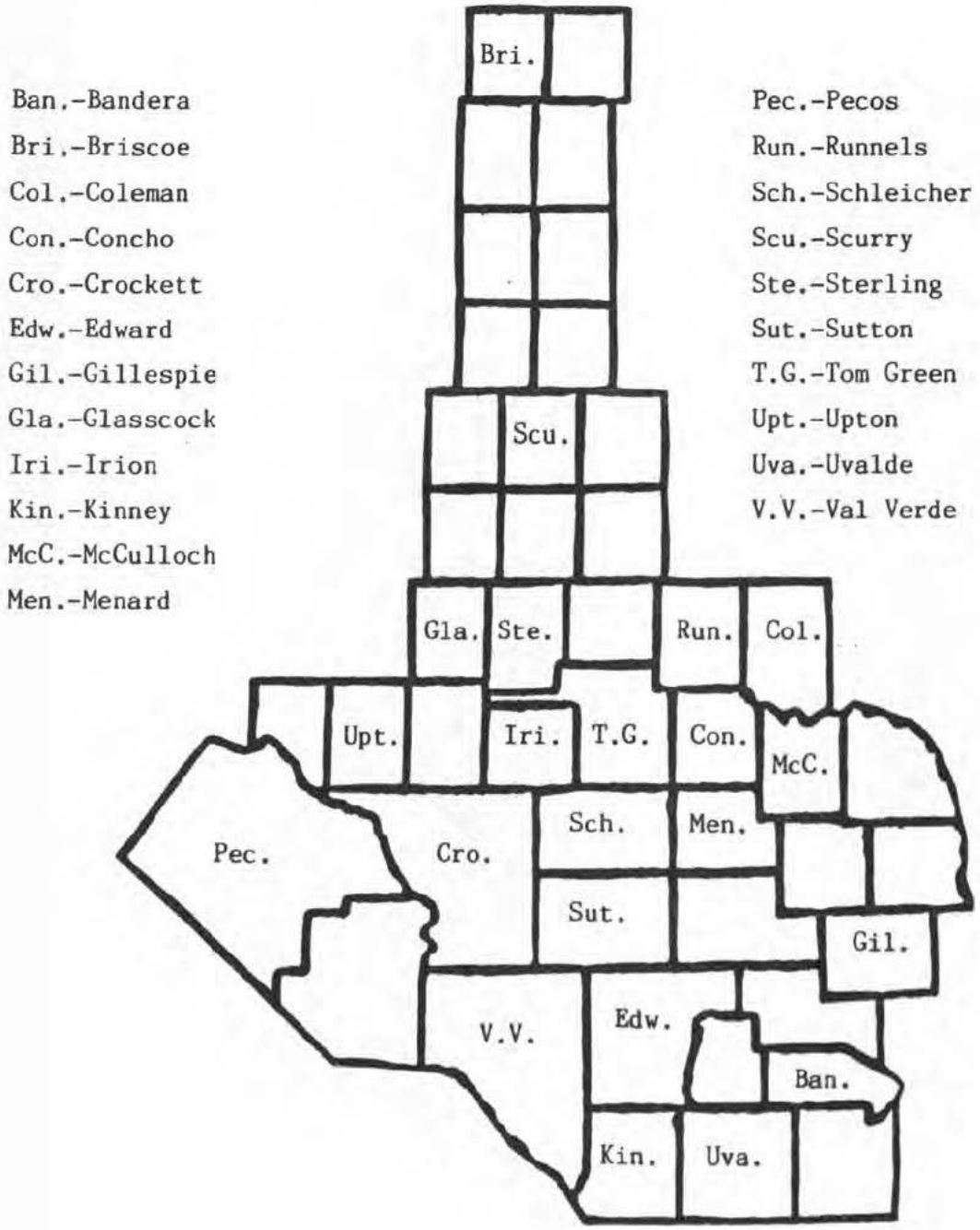
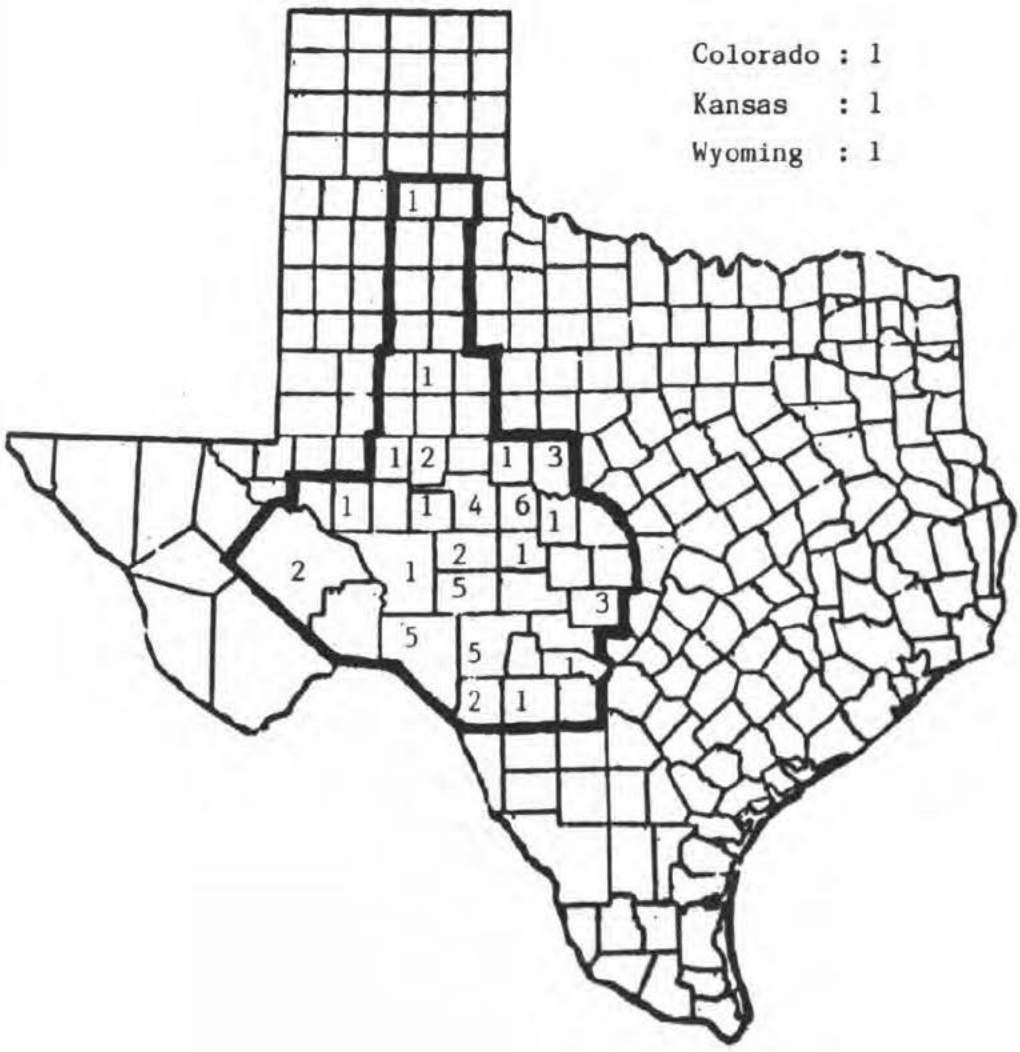


FIGURE 2 - Flocks Tested for Bunyavirus Antibody on a County-by-County Basis



2. Sodium phosphate dibasic ($NaHPO_4$) ----0.73 gm.

3. Potassium phosphate dibasic (KH_2PO_4)--0.20 gm.

4. Potassium chloride (KCl)----------3.00 gm.

5. Glucose ----------20.00 gm.

The solution was sterilized by using a 0.2 micron disposable filter assembly (Acrodisc, Gelman Ann Arbor, Michigan). It was then stored at room temperature. PBS (1x) was prepared by adding 100 ml of 10x PBS to 900 ml of autoclave-sterilized (121C, 15 psi, 15 min.) distilled water.

Antibiotics - A mixture of penicillin, neomycin, and streptomycin (PNS) was used, with the following proportions:

1. One million units of penicillin G potassium (Pfizer, New York, NY).

2. 1 gm of neomycin sulfate (Upjohn, Kalamazoo, Michigan).

3. 1 gm of streptomycin sulfate (Pfzier, New York, NY).

PNS was prepared by adding the components to 100 ml of sterilized 1x PBS. It was dispensed in 2 ml aliquots and stored at -20C. Five million units of mycostatin (Sigma, St. Louis, MO) were dissolved in 500 ml sterile distilled water, dispensed in 5 ml aliquots, and stored at 4C.

Trypsin - A 0.25% dilution of trypsin was prepared by adding 100 ml of 2.5% (10x) trypsin solution (GIBCO, Grand Island, NY) to 900 ml of sterilized 1x PBS. The pH was adjusted to 8.2-8.4 and the solution was dispensed in 10 ml aliquots and stored at -20C.

Vero cell culture - Vero cells, at the 147th and 148th passage levels were obtained from Dr. S. McConnell, College of Veterinary Medicine, Texas A&M University, College Station, Texas. Monolayer

cultures of Vero cells were initially grown at 36C with 5% CO_2 in 75 cm^2 tissue culture flasks (Falcon, Oxnard, CA or Corning, Corning, New York). The growth medium employed in these studies was sterile Eagle Minimum Essential Medium (EMEM) [GIBCO, Chagrin Falls, Ohio] containing Earle's Balanced Salt solution supplemented with the following:

1. 10% fetal bovine serum (FBS) [GIBCO, Grand Island, NY].
2. 1% non-essential amino acid (NEAA) [100x concentrate, GIBCO, Grand Island, NY].
3. 1% sodium pyruvate (NA-P) [100x concentrate, GIBCO, Grand Island, NY].
4. 1% PNS solution.
5. 1% mycostatin suspension.

The pH was adjusted to 7.0-7.2 with 4.4% sodium bicarbonate solution. Vero cell monolayers were maintained in the same medium using 2% horse serum (HS) [obtained from Texas A&M Agricultural Research and Extension Center at San Angelo, Texas] to replace the 10% FBS used to initiate growth. Vero cells were harvested by using trypsin digestion. Storage of the cells was accomplished by suspending them in an antibiotic-free growth medium containing 10% dimethyl sulfoxide (DMSO). Aliquots of the cell suspension were stored at -90C in liquid nitrogen. Seitz filtration was utilized to sterilize all cell culture media.

Virus propagation - Bunyavirus strain 7856, isolated in 1981 from a sick ram near Mertzon, Texas (21), was inoculated onto three-day-old Vero cell monolayers and propagated at 36C with 5% CO_2

atmosphere in 75 cm^2 tissue culture flasks. Maintenance medium was used to maintain the viability of the Vero cell culture to used for viral propagation. The flasks were incubated for 5-7 days, until cytopathic effects (CPE), which is the induction of morphologic changes in the cell cultures, as the viruses replicate) were noted in the Vero cell monolayers. Then, the virus-infected cells in the maintenance medium were harvested and stored in aliquots at -78C.

Virus titration - To determine the viral pool concentration, 0.5 ml of the virus-infected cells in maintenance medium was diluted serially ten-fold in EMEM. Three aliquots of 0.2 ml of each dilution were inoculated onto three-day-old Vero cell monolayers propagated on the wall of 160x150 mm culture tubes (Kimble) at 35C in a tissue culture rotator (Lab-Line, Melrose Park, IL). Three aliquots of each dilution were inoculated onto monolayers in 0.2 ml amount respectively. Enumeration of the virus using the 50% Tissue Culture Infective Dose (TCID 50) method of titering as decribed by Charles H. Cunningham (3) and L. J. Reed and H. Meunch (24) was employed.

Antiserum preparation and virus reisolation - Two milliliters of 2 x 10^4 TCID 50 virus were injected into two 8-month-old Rambouillet lambs (AF8C and AF84) via the jugular vein. AF8C and AF84 were reinoculated subcutaneously a month later and two additional lambs (AF76 and AF88) were inoculated in an identical manner. Rectal temperatures and 10 ml blood samples were obtained daily, beginning with the day of inoculation and continuing 21 days postinoculation (PI). Buffy coats of the blood samples were

inoculated onto three-day-old Vero cell monolayers propagated at 36C in 25 cm2 tissue culture flasks (Falcon, Oxnard, CA) to attempt virus reisolation. Blind passages of the cultures were conducted if necessary. Serum samples were obtained on alternate days beginning on day PI-1 and continuing to day PI-21. Serum samples were also obtained on days PI-25, PI-30, PI-50, and PI-198.

Neutralization test - Beta procedure virus neutralization tests (28) were used for the bunyavirus neutralizing antibody survey and were conducted as follows:

1. Sheep serum was inactivated by heating it at 56C for 30 min. (7, 22) to destroy nonspecific inhibitors of viral infectivity.
2. 0.4 ml of test serum was diluted 1:1 with 0.4 ml of EMEM in a sterile test tube. 0.4 ml of the mixture was then discorded.
3. 0.4 ml of EMEM diluted Bunyavirus at a concentration of 10 TCID 50 was added aseptically to the serum dilution tube and mixed. The mixture was incubated at 35C for 1 hour.
4. 0.2 ml of the serum-virus mixture was layered onto each of the three Vero cell monolayers and incubated at 35C for 1 hour.
5. Maintenance medium was then added to each Vero cell monolayer and the tubes were incubated in a tissue culture rotator at 35C for 6 days.
6. Observations of CPE were made on the third day through the sixth day postinoculation.

7. Accompanying each test, a positive serum and a negative serum control were tested as controls.

Results

The two sheep (AF84 and AF8C) which had been inoculated intravenously via the jugular vein demonstrated no body temperature elevation. Virus was not reisolated from buffy coats and no elevation in antibody levels was observed during the 30 days following inoculation.

Of the four sheep inoculated subcutaneously, two (AF76 and AF88) had elevated body temperatures on day PI-1 (Figure 3). No clinical symptoms were observed. Virus was reisolated on the second passage from buffy coats of AF84, AF8C, and AF76 on days PI-1 and PI-2. The virus could not be reisolated from buffy coats of AF88.

A seropositive reaction to Bunyavirus was first detected on PI-8 in sheep AF8C after subcutaneous inoculation. Although, all of the sera showed a positive reaction on day PI-14, three sheep showed a negative reaction on days PI-16, PI-18, and PI-20 (Table 1). All sera were consistently positive from day PI-21 to day PI-198 (the end of this study). Throughout this study, each serum sample was diluted 1:1 with EMEM and tested in triplicate. It was regarded as positive if the diluted serum prevented CPE in Vero cell monolayers in two or more of the three replicates.

From Texas sheep, 70 serum samples of a total of 366 serum samples collected in 1981 showed positive reactions (19.1%). All of the sheep sampled from six flocks were seropositive, while all those sampled from 16 other flocks were seronegative (Table 2). The total number of positive samples, the total number of serum samples, and the percentage of seropositive samples from 22 Texas counties and from three other states are shown in Figure 4.

FIGURE 3 - Body Temperature Fluctuations in Sheep Experimentally Infected with Bunyavirus Via Subcutaneous Route

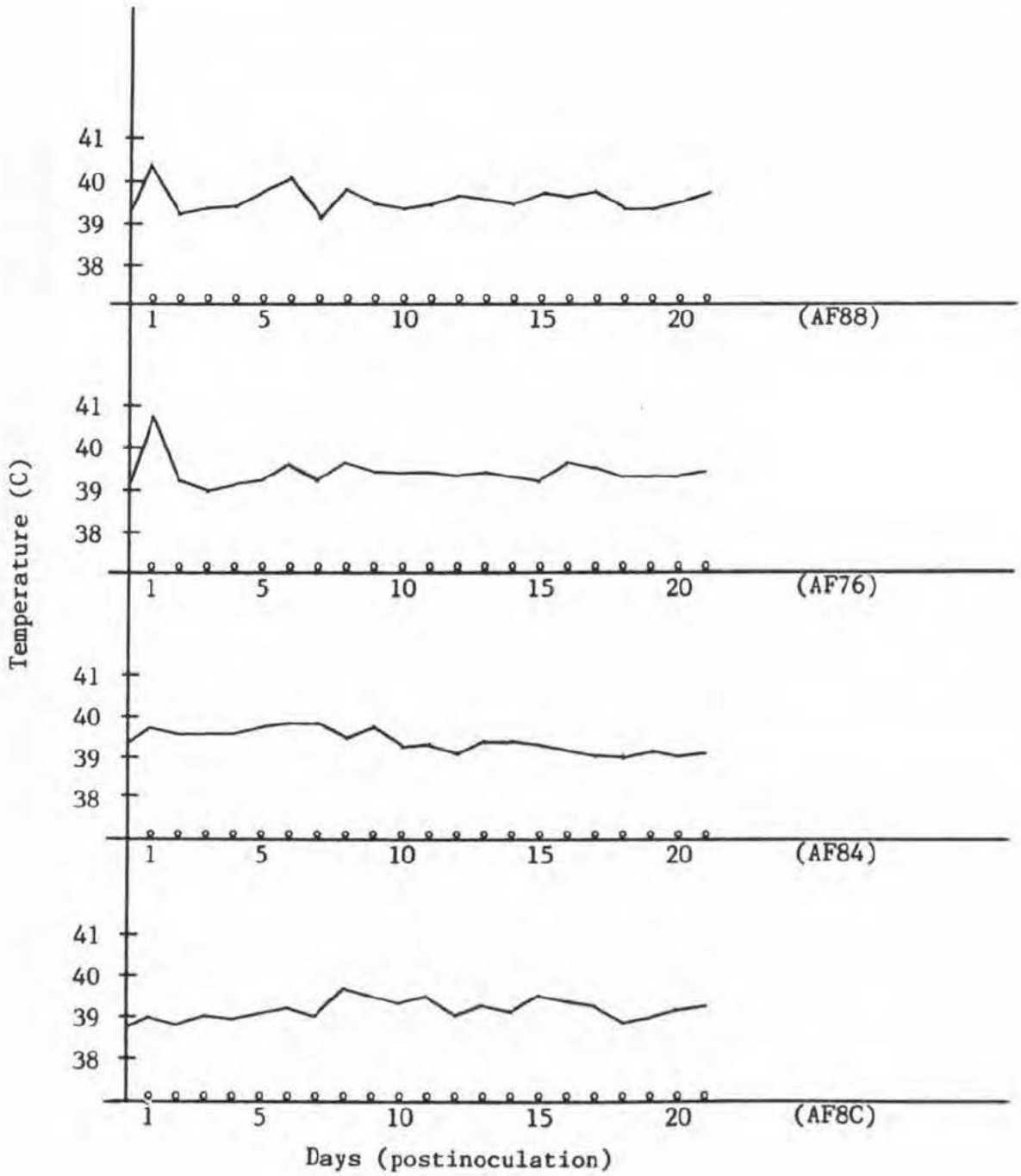


TABLE 1 - Number of Seropositive Reactions to Bunyavirus of Four Experimentally Infected Sheep[@]

Days Postinoculation	AF88	AF76	AF84	AF8C
2	0	0	0	0
4	0	0	0	0
6	0	0	0	0
8	0	0	0	2
10	0	1	1	0
12	0	3	1	2
14	2	3	2	2
16	1	2	1	2
18	2	2	0	1
20	3	3	0	2
21	2	2	2	2
25	3	3	3	3
30	3	3	3	3
50	3	3	3	3
198	3	3	2	3

[@] Each Serum was Diluted to 1:1 with EMEM and Each Serum was Tested in Triplicate

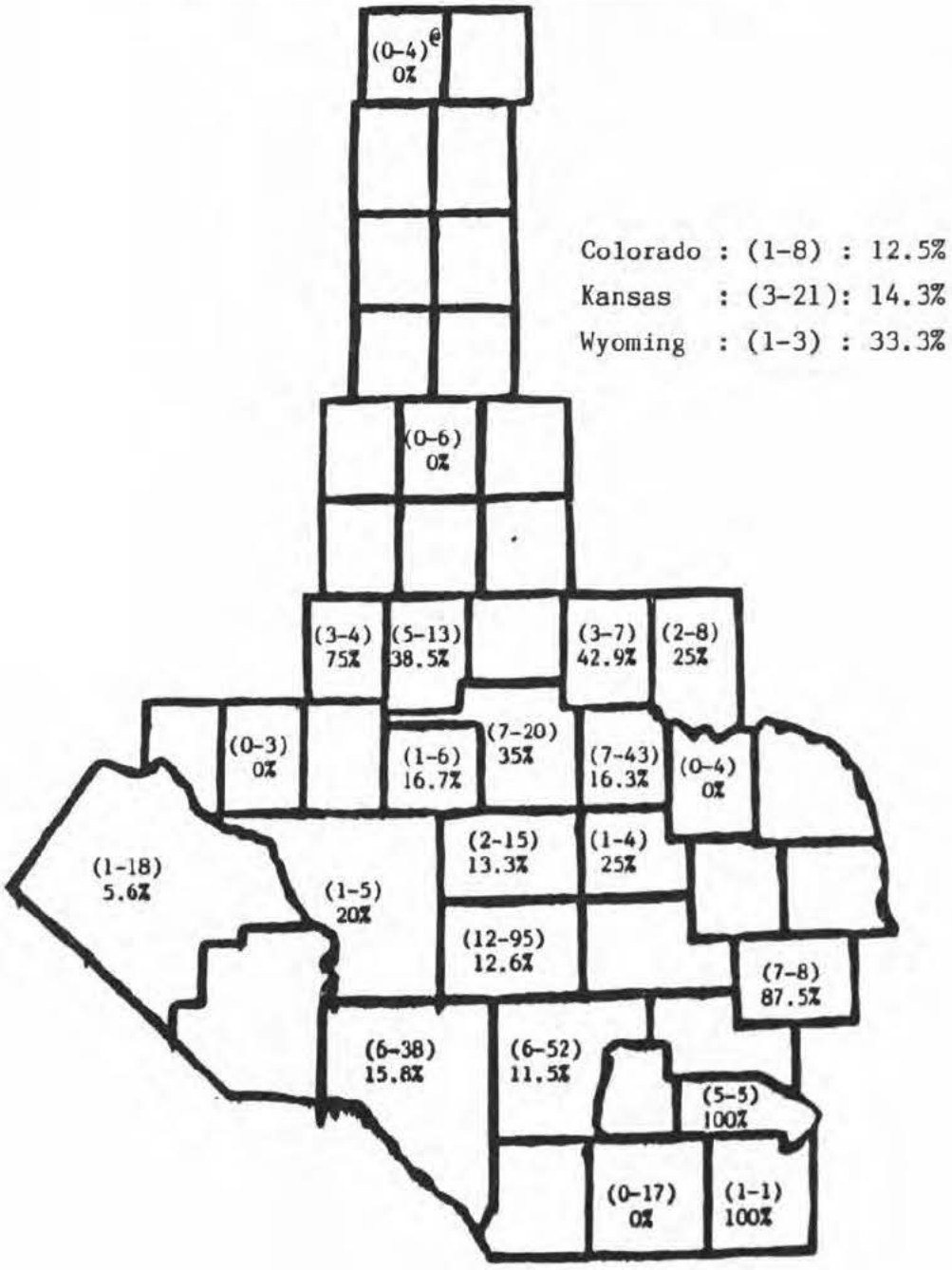
TABLE 2 - The Incidence of Seropositivity to Bunyavirus on a Flock-by-Flock Basis in 1981

Flock	County	Number of Animals Seropositive	Number of Animals Tested	Percentage of Tested Animals Positive
Ban-1	Bandera	5	5	100%
Bri-1	Briscoe	0	4	0%
Col-1	Coleman	0	2	0%
Col-2	Coleman	0	2	0%
Col-3	Coleman	2	4	50%
Con-1	Concho	0	2	0%
Con-2	Concho	0	5	0%
Con-3	Concho	2	10	20%
Con-4	Concho	0	7	0%
Con-5	Concho	1	3	33.3%
Con-6	Concho	4	16	25%
Cro-1	Crockett	1	5	20%
Edw-1	Edward	0	6	0%
Edw-2	Edward	0	8	0%
Edw-3	Edward	4	4	100%
Edw-4	Edward	1	10	10%
Edw-5	Edward	1	24	4.2%
Gil-1	Gillespie	3	3	100%
Gil-2	Gillespie	3	3	100%
Gil-3	Gillespie	1	2	50%
Gla-1	Glasscock	3	4	75%
Iri-1	Irion	1	6	16.7%
Kin-1	Kinney	0	4	0%
Kin-2	Kinney	0	13	0%
McC-1	McCulloch	0	4	0%
Men-1	Menard	1	4	25%
Pec-1	Pecos	0	8	0%
Pec-2	Pecos	1	10	10%

TABLE 2 - Continued

Run-1	Runnels	3	7	42.9%
Sch-1	Schleicher	1	5	20%
Sch-2	Schleicher	1	10	10%
Scu-1	Scurry	0	6	0%
Ste-1	Sterling	1	5	20%
Ste-2	Sterling	4	8	50%
Sut-1	Sutton	2	5	40%
Sut-2	Sutton	3	12	25%
Sut-3	Sutton	3	26	11.5%
Sut-4	Sutton	3	8	37.5%
Sut-5	Sutton	1	34	2.9%
T.G.-1	Tom Green	5	5	100%
T.G.-2	Tom Green	1	2	50%
T.G.-3	Tom Green	0	5	0%
T.G.-4	Tom Green	1	8	12.5%
Upt-1	Upton	0	3	0%
Uva-1	Uvalde	1	1	100%
V.V.-1	Val Verde	0	11	0%
V.V.-2	Val Verde	2	7	28.6%
V.V.-3	Val Verde	1	7	14.3%
V.V.-4	Val Verde	2	6	33.3%
V.V.-5	Val Verde	1	7	14.3%
Total in Texas		70	366	19.1%
Colorado		1	8	12.5%
Kansas		3	21	14.3%
Wyoming		1	3	33.3%

FIGURE 4 - Total Number of Seropositive Samples, Total Number of Samples, and Percentage of Positive Reactions from Counties in Texas and from Three Other States



@ (Positive Reaction - Total Samples)
Positive Percentage

Two previously tested flocks were sampled again in 1986. Flock T.G.-1, which previously showed 100% seropositivity, when resampled, had four of 40 animals testing seropositive , including a 3-year-old ram (of three tested) and three 4-month-old ewe lambs (of 30 tested). None of the seven ram lambs tested were seropositive. Flock T.G.-3, which had previously been seronegative, yielded two seropositive samples, of 40 tested. The samples collected in 1981 from one McCulloch County flock were seronegative, while another flock sampled in 1986 revealed one seropositive reactor among the 22 ewes sampled. The two samples from 4-year-old ewes were also positive.

Discussion

The primary goal of this thesis research was to survey the geographic distribution of bunyavirus neutralizing antibodies among the sheep population in Texas. In addition, an immunological study of four experimentally infected sheep was included as part of the current study. Neutralizing antibodies against the homologous Bunyamwera virus developed in all four experimentally inoculated sheep. A febrile response occurred on day PI-1 in two of the four sheep and the virus was reisolated at the second passage on day PI-1 and day PI-2 from three of the four sheep. This demonstrates that the virus is capable of producing a transient febrile reaction with the production of specific antibodies. A similar pattern of infection has been described in other animals by Kokernot et al. (17) who stated; "a monkey showed no clinical signs, but had a detectable viremia on the postinoculation day of the inoculation of a strain of Bunyamwera virus" and "the first passage of the virus never caused sickness in adult mice". They also pointed out that the neutralizing antibody titer that developed against a strain of Bunyamwera virus during the acute phase was much lower than that developed during convalescent phase. A seropositive reaction was detected first on day PI-8 in the present study, which was similar to the time required for development of neutralizing antibody titer demonstrated by Kokernot et al. (17) in the previous study. Three sheep showed negative serological reactions after day PI-14 (Table 1). The negative results might have been due to the serum samples having been frozen and thawed several times.

Bunyamwera virus has been isolated from domestic animals and other mammals (21) and significant levels of neutralizing antibodies have been detected and studied in cattle, sheep, goats, donkeys, dogs, and human beings by Kokernot et al. (18), in snakes and turtles by Whitney et al. (32), and in deer by Hoff et al. (9) and Issel et al. (15). On the other hand, large doses of Cache Valley virus neither produced a viremia in chicks nor demonstrable neutralizing antibodies in the sera 30 days postinoculation (10). Kokernot et al. (16) also reported that serum antibodies protective against Bunyamwera virus were not detected in serum samples from 240 immature birds.

The absence of body temperature elevation, viremia, and detectable neutralizing antibodies in AF84 and AF8C after jugular vein injection indicate intravenous administration did not induce the immune response in those sheep. Subcutaneous injection was shown to be more effective at provoking the immune response. From these results it appears that virus injected into the blood is rapidly cleared, whereas subcutaneously injected virus may not be removed as rapidly from the body and produces a more effective immune stimulus. The virus probably produces slight damage to tissue, leading to elevation of body temperature.

The geographic distribution of bunyavirus neutralizing antibodies in the sheep population of Texas was studied by testing 366 serum samples collected in 1981 from rams placed in the annual Sonora Ram Test. The Ram Test begins every year in October and ends in March the following year at the testing station near Sonora, Texas. These yearling rams originated from nearly all the counties producing sheep in Texas and included flocks from three other states. As shown in Table 2 and Figure 4, the presence of bunyavirus neutralizing antibodies in the

sheep population of Texas was geographically widespread but the percentage of infected animals was low (70/366, 19.1%). Sera protective against Bunyamwera virus have been reported as rare in three species of domestic quadrupeds and to be completely absent among dogs in Tongaland (18), but high in the donkeys of Tongaland (76.2%) (18) and the deer of Texas (100%) and Wisconsin (72-100%) (15).

Previous studies showed that a high percentage of animals in the vicinity of rivers and lakes demonstrated neutralizing antibodies against Bunyamwera virus (18) and that mosquitoes collected from those areas were sources for Bunyamwera virus isolation (2). The percentage of positive sera from sheep, goats, and cattle have been compared relative to different geographic zones including mountain areas (8.8%), river areas (13.4%), and coastal plain areas (13.5%) (18). In general, the southern and eastern parts of Texas have a wetter climate than that of west Texas. It is possible that such a difference might influence mosquito populations leading to an increased incidence of infection in wildlife and domestic animals around those areas conducive to mosquito growth. In the present study, flock T.G.-1, adjacent to flock T.G.-3 and closer to a lake than flock T.G.-3, demonstrated a higher percentage of seropositive reaction in both years tested (1981, 5/5, 100%. 1986, 4/40, 10%) than that of flock T.G.-3 (1981, 0/5, 0%. 1986, 2/40, 5%). It was impossible to obtain with certainty the ranges of mosquitoes and the actual geographic zone of the ranches from which sheep sera were donated. A relationship between geographic zone and percentage of animals with protective serum could therefore not be determined.

Older human and animal populations, have a higher percentage of individuals yielding protective sera against Bunyamwera virus than

younger ones as demonstrated in several earlier studies (9, 16, 18). This is perhaps due to a greater opportunity for these individuals to become exposed to the viruses because of their age. It was not possible to determine the relationships between the age of the sheep and the presence of neutralizing antibodies against Bunyavirus because the age of most of the sheep ranged from four to 14 months old. However, in sheep that were three years or older, the percentage of seropositive reactions was 60% (3/5). This percentage is obviously greater than that obtained for the sheep population as a whole indicating a possible age relationships.

The white-tailed deer (Odocoileus virginianus), one of the most widely distributed native mammalian species of North America, has been evaluated in Texas and Wisconsin for the presence of neutralizing antibodies against Bunyamwera virus. In Texas, in 1970-1972, 81% (114/140) of the animals studied was seropositive (9) and 100% in both 1963 and 1969 (37/37 and 44/44, respectively) was seropositive (15). In Wisconsin, the incidence varied from 72-100% in 1963 and 42-79% in 1969, respectively, depending on the geographic zone (15). The high percentage of positive sera against Bunyamwera virus among deer as shown in the earlier studies and the low percentage among sheep as demonstrated in the present study as well as the low percentage among domestic quadrupeds in Tongaland (18) suggest that wildlife is more commonly infected with Bunyamwera virus, and hence, might play a more important role in its natural history. However, it was possible that the tested deer were much older than the tested domestic animals.

The detection of bunyavirus neutralizing antibodies in the serum samples of ram lambs from three other states demonstrated that the

infection of Bunyavirus was not limited to Texas in 1981. Seropositive reaction of samples from three 4-month-old ewe lambs in 1986 indicates that infection by Bunyavirus still exists among the sheep population in Texas. The presence of bunyavirus neutralizing antibodies in the serum samples of a 3-year-old ram and two 4-year-old ewes indicates that the infection could have occurred as early as 1982 or as late as 1986 because bunyavirus neutralizing antibodies, once formed, last for at least 198 days (Table 1). Therefore, it is possible that infections of Bunyavirus have occurred in Texas every year between 1982 and 1986.

This study demonstrated that Bunyavirus infection in Texas was geographically widespread but that the percentage of infected animal was low. The virus was shown to be of minimal pathogenicity to sheep but the neutralizing antibodies produced were detectable for at least 198 days. This virus has previously been shown to cause nervous system disturbances and death of conventional and gnotobiotic lambs and kids (21), but not in adult animals.

The present study is the first attempt to examine the extent of Bunyavirus infection among sheep in the United States. Many aspects of this virus and its role in livestock and wildlife morbidity are unknown. Future studies might focus upon such features as the clinical signs of naturally infected animals, the role of geographic zones, animal age and seasonal changes, mosquito vectors, economic losses from the infection, relationships with other recognized pathogens of ruminants, and also preventive measures.

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